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In vitro antiplasmodial activity and phytochemical screening of crude extracts of *Senna sieberiana* DC. (Fabaceae), plant used in traditional medicine

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Abstract

Medicinal plants contain many phytocompounds whose properties are recognized to prevent or treat various diseases including malaria. This study was conducted to evaluate the antiplasmodial activity and determine the phytochemical composition of different extracts of the leaves of *Senna sieberiana* DC., a plant used in traditional medicine to treat malaria. The schizonticidal activity of the extracts was measured *in vitro* by the Syber Green fluorimetric method, on clinical isolates and on the reference strains NF54 and K1 of *Plasmodium falciparum*. Of the three types of extracts (macerated, decocted and methanolic) tested, the methanolic extract of the leaves was found to be highly active both on clinical isolates and on the strains NF54 and K1 of *P. falciparum* with IC_{50} values ≤ 5 µg/mL. The tested leaf extracts of *Senna sieberiana* all contained polyterpenes and sterols, polyphenols, flavonoids, alkaloids and tannins. The methanolic extract of this plant could be a candidate for the formulation of an effective MTA against malaria.

Keywords: Antiplasmodial activity; *Senna sieberiana*; Phytochemical; Malaria

1. Introduction

Malaria is a potentially fatal endemic disease in hot and humid tropical areas. It is caused by a hematozoan of the genus *Plasmodium* and transmitted by the bite of a mosquito, the female *Anopheles* [1]. Five species of *Plasmodium* are capable of infesting humans, *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*. Among these five species, *P. falciparum* is the most widespread in sub-Saharan Africa and the deadliest [2]. In 2022, the WHO recorded 249 million cases of malaria worldwide, including 608,000 deaths due to this disease. More than 94% of cases and 95% of deaths related to this disease in 2022 were located in sub-Saharan Africa [3]. In Côte d'Ivoire, according to the PNLP, in 2022, the number of malaria cases was estimated at 7,342,925 and the number of deaths at 1,555. It is the leading cause of morbidity and mortality in children under 5 years of age [4]. Despite the progress made in the fight against this pandemic, the emergence of strains of *P. falciparum* resistant to current antimalarials remains a threat to achieving the goal of eliminating malaria by 2030. Indeed, over the past decade, partial resistance to artemisinin has emerged independently in parts of Southeast Asia, South America, and East Africa, affecting the efficacy of artemisinin-based combination therapy, the main class of antimalarials used worldwide [5]. Given that few options are available for antimalarial drugs,

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it is necessary to research and develop new antimalarial drugs that are effective against resistant strains. In this study, we propose to evaluate the antiplasmodial activity of different extracts of *Senna sieberiana* DC. (Fabaceae) used in traditional medicine to treat malaria.

2. Materials and methods

2.1. Preparation of extracts

The plant material, composed of *Senna sieberiana* leaves, was harvested in the locality of Bouaké in central Côte d'Ivoire in the month of April. The leaves of the plant were dried in air conditioning (21 °C) for two weeks and then pulverized to obtain a fine powder. Three extraction methods were carried out in this work: decoction and aqueous maceration inspired by traditional preparations then extraction by organic solvents of increasing polarity to optimize the extraction and concentrate the compounds.

The decoction was made by dissolving 25 g of plant powder in 250 mL of distilled water and the whole was heated to 100 °C for 15 min. The mixture was filtered and dried in an oven at 45 °C.

The aqueous macerate was obtained by dissolving 25 g of plant powder in 250 mL of distilled water in an Erlenmeyer flask under mechanical stirring (175 rpm) for 24 h. The mixture was filtered and dried in an oven at 45 °C.

For the successive extraction with solvents of increasing polarity, 25 g of each powder was macerated in 250 mL of dichloromethane under mechanical stirring for 24 h. After filtration, the residual marc was dried at room temperature, weighed, then taken up in a volume of 250 mL of methanol and macerated again under mechanical stirring for 24 h. The mixture was filtered, concentrated on a rotary evaporator at 40° C and dried in an oven at 45 °C.

2.2. Phytochemical screening of the extracts

Phytochemical screening of the extracts of *S. sieberiana* was carried out for the search for secondary metabolites. The analyses were carried out according to the method of standard tube colorimetric tests described in the works of Zirihi *et al.* (2003) [6], Békro *et al.* (2007), [7] and N'Guessan *et al.* (2009) [8].

2.2.1. Highlighting sterols and polyterpenes

0.1 g of extract was dissolved hot in 1 mL of acetic anhydride in a capsule and then taken up in a test tube into which 0.5 mL of concentrated H_2SO_4 was poured. The appearance at the interphase of a purple or purple ring, turning blue then green, revealed the presence of sterols and triterpenes.

2.2.2. Highlighting saponins

2 mL of extract in a test tube were shaken vigorously for 35 s then left to stand. The persistence of a foam more than 1 cm high after 15 min indicated the presence of saponins.

2.2.3. Highlighting alkaloids

6 mL of extract were evaporated to dryness. The residue was taken up in 6 mL of alcohol at 60 °C. 2 drops of Dragendorff's reagent were added to the alcoholic solution. The observation of a precipitate or an orange coloration indicates a positive reaction.

2.2.4. Highlighting polyphenols

To 2 mL of extracts, a drop of 2% alcoholic ferric chloride solution was added. The appearance of a more or less dark blue-black or green coloration indicates a positive reaction.

2.2.5. Highlighting flavonoids

In a tube containing 3 mL of the extract solution, a few drops of a 10% NaOH solution were added. A yellow-orange color characterizes the presence of flavonoids.

2.2.6. Highlighting tannins

To 1 mL of extract contained in a test tube were added 2 mL of water then one to two drops of 1% ferric chloride. The appearance of a blue, blue-black or black color indicates the presence of gallic tannins; green or dark green color indicates the presence of catechic tannins.

2.3. Evaluation of *in vitro* antiplasmodial activity

2.3.1. Preparation of test solutions of extracts and controls

The stock solutions of the crude plant extracts were prepared at 1 mg/mL. 10 mg of extract were dissolved in 10 mL of RPMI 1640 medium (Roswell Park Memorial Institute 1640) for the aqueous extracts and in 1 mL of dimethyl sulfoxide (DMSO) adjusted to 10 mL with RPMI 1640 medium for the methanolic extract. After vortexing, the mixture was filtered through 0.45 µm millipore filters. The filtrates obtained were stored in a freezer at +4°C and were used for antiplasmodial tests [9].

2.3.2. Preparation of culture media (RPMI 1640)

The culture medium used for *in vitro* tests was RPMI 1640 supplemented with 25 mM HEPES, 5% sodium bicarbonate solution and 10% human serum (O). It was prepared by dissolving 10.4 g of the powdered material in one liter of distilled water and then autoclaved at 121 °C for 15 min [9].

2.3.3. Inoculum preparation

Five clinical isolates of *P. falciparum* coded W6331, W6401, W6424, W6640 and W6870, collected from patients with uncomplicated malaria with their consent at the Urban and Community Health Training of Yopougon Wassakara (Abidjan, Côte d'Ivoire) and two reference strains, chloroquine-resistant strain K1 and chloroquine-sensitive strain NF54 of *P. falciparum* provided by the Cytology Laboratory of the Swiss Center for Scientific Research (CSRS) in Côte d'Ivoire. Study approval was issued by the National Ethical Committee and Research of Côte d'Ivoire. The parasitized blood samples with an initial parasitemia of 0.1 to 0.3% were washed three times with RPMI 1640 culture medium by centrifugation to remove serum and white blood cells. The parasitized red blood cell (RBC) pellet was suspended in RPMI medium, this time enriched with 0.5% Albumax, to obtain an inoculum with a hematocrit of 5% [10].

2.3.4. Antiplasmodial activity test

The *in vitro* antiplasmodial activity tests used in this work were performed according to the WHO recommended Rieckmann Microtest. The inhibition of *P. falciparum* erythrocyte schizogony was measured using the SYBR Green method [9]. This method measures and quantifies the ability of the drug to inhibit the growth of *P. falciparum* at the trophozoite stage using SYBR Green, which is intercalated between the DNA bases and emits fluorescence whose intensity is proportional to the DNA in the medium, which is itself proportional to the number of parasites. To perform the tests, 40 µL of test extract at 1 mg/mL and 160 µL of culture medium were introduced into the wells of the first row of a 96-well microplate. After homogenization, a half-dilution is carried out from the wells of the 2nd row and 100 µL of parasite inoculum are added to all the wells giving in duplicate, a dilution range from 100 to 1.56 µg/mL for plant extracts and from 200 to 3.12 nM for chloroquine (CQ) used as the reference antimalarial molecule [10]. The microplates were placed in a candle bell then in the incubator for 72 h at 37 °C. After 72 hours of incubation, the red blood cells were lysed by adding a lysis solution containing 0.2 µL of SYBR Green per mL of lysis buffer. After 1 h of incubation, the reading was made with a spectrofluorometer at 535 nm.

2.3.5. Data processing

The analysis of the fluorescence data was done by the software "ICESTIMATOR version 1.2. [11] allowing the determination of the 50% Inhibitory Concentration (IC_{50}). The antimalarial activity of the extracts was defined from the IC_{50} values obtained, according to the classification of Jansen *et al*. (2012) [12]. Thus an IC_{50} value ≤ 5 µg/mL was classified as highly active. Extracts with values 5 µg/mL ≤ IC_{50} ≤ 15 µg/mL were considered to have promising activity. Extracts with values 15 µg/mL ≤ IC_{50} ≤ 50 µg/mL were considered moderately active and those with IC_{50} values ≥ 50 µg/mL were considered inactive.

3. Results

3.1. Antiplasmodial activity

The results of the measurement of the *in vitro* antiplasmodial activity of the extracts are reported in Tables 1 and 2. Analysis of the results showed that the tested extracts are active on both clinical strains (Table 1) and reference strains (Table 2) of *P. falciparum*. The tested extracts have promising antiplasmodial activity on clinical strains and reference strains of *P. falciparum* with IC_{50} values ranging from 1.42±0.19 to 13.77±1.54 µg/mL. However, the best antiplasmodial activity of the extracts was obtained with the methanolic extract of *S. sieberiana* leaves which was highly active on all the tested isolates and on the reference strains NF54 and K1 with IC_{50} values ≤ 5 µg/mL.

Table 1 Antiplasmodial activity of *Senna sieberiana* leaf extracts on clinical isolates of *P. falciparum*

Plant species	**Extracts**	**IC_{50} of clinical isolates of *P. falciparum* (µg/mL)**				
		W6331	W6401	W6424	W6640	W6870
Senna sieberiana	Mac.	7.23±1.4	13.77±1.54	10.06±1.64	9.39±1.68	13,15±1,63
	Dec.	12.45±1.45	8.17±0.77	7.42±0.8	7.39±0.65	7,77±0,83
	MeOH	1. 62±0.2	1.51±0.76	1.42±0.19	3.94±0.36	4,46±0,16
Chloroquine (nM)		7,8±0,19	7.2±0.14	13.3±0.23	ND	11.2±0.17

Dec = Decocted ; Mac = Aqueous maceration ; MeOH = methanolic extract ; ND = Not Determined.
Values in bold represent IC_{50} values ≤ 5 µg/mL

Table 2 Antiplasmodial activity of *Senna sieberiana* leaf extracts on reference strains NF54 and K1 of *P. falciparum*

Plant species	**Extracts**	**IC_{50} of *P. falciparum* reference strains (µg/mL)**	
		NF54	**K1**
Senna sieberiana	Mac.	ND	ND
	Dec.	15.58±0.44	18.27±0.96
	MeOH	04.54±0.94	5.34±0.01
Chloroquine (nM)		16,65±1,21	124.64±3.86

Dec = Decocted; Mac = Aqueous maceration; MeOH = methanolic extract; ND = Not Determined.
Values in bold represent IC_{50} values ≤ 5 µg/mL

3.2. Phytochemical screening

Phytochemical screening conducted to identify phytochemical groups present in the extracts and likely to be responsible for the observed activities revealed the presence of several secondary metabolites as shown in Table 3. Analysis of these results highlighted the presence of polyphenols, flavonoids, alkaloids, tannins, polyterpenes and sterols.

Table 3 Class of phytochemical groups present in extracts of *Senna sieberiana* leaves

Plant species	**Extracts**	**Polyterpenes and sterols**	**Polyphenols**	**Flavonoids**	**Tannins**	**Alkaloids**	**Saponins**
Senna sieberiana	Mac.	+	+	+	+	+	+
	Dec.	+	+	+	+	+	+
	MeOH	+	+	+	+	+	-

Dec = Decocted ; Mac = Aqueous maceration ; MeOH = methanolic extract ; + = presence; - = absence

4. Discussion

This study consisted of evaluating the antiplasmodial activity of different extracts of *S. sieberiana* leaves and determining their phytochemical composition. This plant was selected following an ethnobotanical survey on plants with antimalarial potential, carried out among traditional healers and herbalists in the Abidjan district [13].

4.1. Antiplasmodial activity

The tested extracts have promising antiplasmodial activity on clinical strains and reference strains of *P. falciparum*. The best antiplasmodial activity was obtained with the methanolic extract of *S. sieberiana* leaves which was highly active on all the tested isolates and on the reference strains with an IC_{50} of 5.34 µg/mL on K1. These results corroborate those obtained by Aliyu *et al*. (2013) [14] for the evaluation of the antiplasmodial activity, with another method, of this same plant. These authors showed, *in vivo*, a quasi-absence of the parasite after 72 hours of incubation, with the methanol and benzene extracts of leaves of *S. sieberiana*. The anti-plasmodiale activity obtained with the methanolic extract of this plant is located in the same order as that observing with the extracts of *Artemisia annua* [15]. This antiplasmodial activity obtained with the methanolic extract of *S. Siberiana* supports the traditional use of this plant to treat malaria. This result is a high promoter and would be interesting when taking into account additional perspectives for its local recovery.

The fact of associating clinical isolates in this study, responds to the concern of being close to reality; these strains are present in the population targeted by the treatment by medicinal plants. Indeed, the reference strains adapted to laboratory culture, could no longer present the same sensitivity as the reality of the field as similarly indicated by Tuo (2015) [16] and Koffi *et al*. (2020) [10]. The results obtained with the reference strains support those obtained with the clinical isolates. It is therefore interesting to note that the sensitivity profile observed with the crude extracts on the reference strains is identical to that of the clinical isolates. Such a result was also recorded by Tuo (2015) [16] and Koffi *et al*. (2020) [10] who stipulate that freshly harvested clinical isolates could be used to determine the schizonticidal activity of any antimalarial like the laboratory strains.

4.2. Phytochemical screening

Phytochemical screening revealed the presence of polyphenols, flavonoids, alkaloids, tannins, polyterpenes and sterols in the leaves of *S. sieberiana*. Several of these phytocompounds are responsible for various pharmacological properties. The alkaloids, polyphenols, flavonoids and saponins found in the leaves of *S. sieberiana* are known for their antiplasmodial activity [17]. According to these authors, these phytocompounds identified in the methanolic extract of the studied plant play a key role in antiplasmodial activity. These compounds are recognized for their ability to disrupt the metabolism of the *Plasmodium falciparum*, either by inhibiting protein synthesis, or by generating lethal oxidative stress for parasitis. Polyterpenes are attributed with numerous pharmacological properties including analgesic, anti-inflammatory and antiplasmodial activity, particularly with artemisinin, a sesquiterpene effective against resistant forms of malaria [18]. All of these very promising results constitute a scientific validation of the traditional use of this plant in the Ivorian pharmacopoeia in the search for treatment against malaria.

5. Conclusion

This study, which consisted in carrying out the antiplasmodial and phytochemical screening of *S. sieberiana* leaf extracts, confirmed the antimalarial potential of this plant from the ivorian flora. This plant can be used in the preparation of phytomedicines in order to propose alternatives to malaria treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest declarations have been reported.

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